研究論文

Effects of Dietary Sea Squirt (*Halocynthia roretzi*) on Serum and Liver Lipid Levels in Rats

Masashi KAWASAKI

ABSTRACT

To investigate whether or not dietary sea squirt has food and nutritional functions, the effects of sea squirt feeding on serum lipid concentrations and liver lipid contents were examined in rats. Rats were fed a basal diet or experimental diet containing 5%, 10%, or 20% sea squirt muscle. Serum total cholesterol, very-low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL)-cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA) concentrations were gradually reduced by the feeding of an increasing amount of the sea squirt. These serum lipid concentrations in the group that was fed 20% of the sea squirt were significantly lower than those in the group fed the basal diet. Serum high-density lipoprotein (HDL)-cholesterol concentration was not significantly different among the four groups. Liver triglyceride content in the group fed 20% of the sea squirt was significantly lower than that in the group fed the basal diet. Liver cholesterol and phospholipid contents did not differ among the four groups. The serum thiobarbituric acid-reactive substance (TBARS) value was not significantly different among the four groups. The liver TBARS value in the group fed 20% of the sea squirt was significantly higher than that in the group fed the basal diet. These results suggest that the feeding of the dietary sea squirt muscle exerts lowering effects on the levels of serum lipids, such as total cholesterol, (VLDL+LDL)-cholesterol, triglyceride, phospholipid, and NEFA concentrations.

Key words: dietary sea squirt; serum lipid concentration; rat.

INTRODUCTION

Studies on metabolic and nutritional effects of seafoods have been well conducted. Dietary seafoods and their ingredients have been demonstrated to change the lipid profiles of serum and liver in humans and rats. Dietary defatted sea squid decreased the serum total cholesterol concentration and hepatic cholesterol and triglyceride contents.[1] Dietary black sea cucumber[2] and oyster[3] decreased the serum total cholesterol concentration and liver cholesterol content, and elevated the ratio of high-density lipoprotein (HDL)-cholesterol concentration to the total cholesterol concentration of serum. Dietary oyster also decreased serum and liver triglyceride levels.[3] Dietary fish oil, rich in n-3 polyunsaturated fatty acids, has been well documented to suppress the concentrations of serum lipids such as total cholesterol, very-low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL) cholesterol, triglyceride, phospholipid, and nonesterified fatty acid (NEFA) in normal rats,[4,5] and in hyperlipidemic patients[6] or rats.[7,8]

Sea squirt (*Halocynthia roretzi*) is a seafood that lives mainly around the coast of Sanriku. Much study has been done of its biological aspects, but fewer nutritional and food functional studies have been done.

In the present study, to investigate whether or not the dietary sea squirt has food and nutritional functions, the effects of the sea squirt feeding on serum lipid concentrations and liver lipid contents were examined in rats.

Abbreviations: DHA, decosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; TBARS, thiobarbituric acid-reactive substance; VLDL, very-low-density lipoprotein.

MATERIALS AND METHODS

Materials. Fresh sea squirts were obtained from a market. An edible fraction (the muscle parts) was freeze-dried and powdered. The composition of the dried sea squirt muscle was as follows: 35.0% crude protein, 10.1% crude fat, 6.4% crude ash. The water content was 2.9%.

Animals and diets. Male Wistar rats, three weeks of age (Charles River Japan, Inc., Kanagawa, Japan), were individually housed in stainless steel cages and kept in an

air-conditioned room at 22-24℃, with relative humidity at 55 ± 5% in a 12-h light cycle (8:00-20:00). The animals were fed a stock pellet diet (MF; Oriental Yeast Co., Tokyo, Japan) for 3 days, followed by a 20% casein (basal) diet for 4 days. The composition of the basal diet was as follows: 20% casein (Oriental Yeast Co.), 13.2% α-cornstarch (Oriental Yeast Co.), 39.75% cornstarch (Oriental Yeast Co.), 10% sucrose (Nissin Sugar Manufacturing Co., Tokyo, Japan), 5% cellulose powder (Oriental Yeast Co.), 7% soybean oil (Oriental Yeast Co.), 3.5% mineral mixture (AIN 93G composition)[9] (Oriental Yeast Co.), 1% vitamin mixture (AIN 93 composition)[9] (Oriental Yeast Co.), 0.25% choline bitartrate (Wako Pure Chemical Industries, Osaka, Japan), and 0.3% L-cystine (Wako Pure Chemical Industries). Subsequently, the rats were divided into four groups with similar body weights and were fed the basal diet or an experimental diet containing sea squirt muscle, and water ad libitum for 21 days. The experimental diet was prepared by adding 5%, 10%, or 20% of the sea squirt muscle to the basal diet at the expense of cornstarch. Animals were deprived of their diet at 9:00 on day 21 but were given free access to water until they were sacrificed, which was done 4 hours after stopping the diet supply. Blood was collected and left to clot at room temperature so that serum could be obtained. The liver was quickly removed, washed with cold 0.9 % NaCl, blotted on filter paper, and weighed. The serum and liver were stored at -80℃ until analyses were done. Aliquots of the liver were also preserved in methanol and stored at 4℃ until analyses of the lipid contents were performed.

Animal experiment was conducted in accordance with the standards relating to the care and management, etc. of experimental animals (Notification No. 6, March 27, 1980 of the Prime Minister's Office, Japan).

Lipid analyses. The serum total cholesterol, HDL-cholesterol, triglyceride, phospholipid, and NEFA concentrations were measured by using Cholesterol C-test, HDL-Cholesterol-test, Triglyceride G-test, PL-test, and NEFA-test kit (Wako Pure Chemical Industries). The difference between total cholesterol and HDL-cholesterol was regarded as (VLDL+LDL)-cholesterol. Liver total lipids were extracted according to the procedure of Folch *et al.*[10] After portions of the chloroform phase had been dried under nitrogen, the contents of cholesterol,[11] triglyceride,[12] and phospholipid[13] were determined. Serum thiobarbituric acid-reactive substance (TBARS) value was determined using commercial kit purchased from Wako Pure Chemical Industries. Liver TBARS value was measured by according to Mihara *et al.*[14]

Statistical analysis. Results were expressed as mean ± standard errors. Statistical analysis was carried out by one-way analysis of variance followed by Dunnett's multiple test.

RESULTS

There were no differences in food intake or body weight gain during the 21 days of experimental feeding, and liver weight also did not differ among the four groups (Table 1).

The serum lipid (total cholesterol, HDL-cholesterol, (VLDL+LDL)-cholesterol, triglyceride, phospholipid, and NEFA) concentrations are illustrated in Figure 1. The serum total cholesterol, (VLDL+LDL)-cholesterol, triglyceride, phospholipid, and NEFA concentrations were reduced in a dose-dependent manner following feeding of increasing amounts of the sea squirt, and these serum lipid concentrations of 20% of the sea squirt diet

Table 1. Initial body weight, food intake, body weight gain, and liver weight in rats fed basal or sea squirt containing diet.[1]

Measurement	Control[2]	5S	10S	20S
Initial body weight (g)	92.3 ± 1.8	92.3 ± 1.6	92.3 ± 1.5	92.3 ± 2.0
Food intake (g/21days)	396.6 ± 11.8	392.5 ± 9.0	380.3 ± 6.8	376.4 ± 10.1
Body weight gain (g/21days)	164.0 ± 5.5	167.3 ± 4.2	161.3 ± 3.7	167.3 ± 6.5
Liver weight (g)	10.6 ± 0.5	10.9 ± 0.6	10.7 ± 0.2	11.2 ± 0.6

[1] Each value represents the mean ± SEM for seven rats.

[2] Definition of group names: Control, basal diet group; 5S, basal diet containing 5% sea squirt muscle group; 10S, basal diet containing 10% sea squirt muscle group; 20S, basal diet containing 20% sea squirt muscle group.

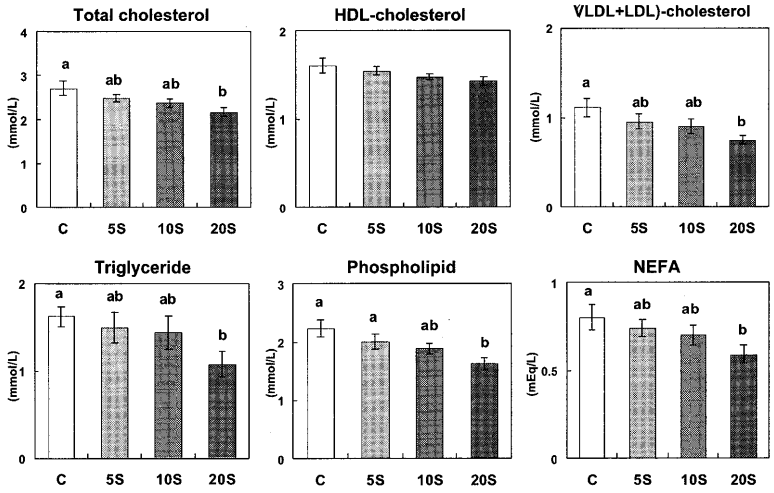

Fig. 1 Serum lipid concentrations in rats fed basal or sea squirt containing diet. Each value represents the mean for seven rats. Vertical bars indicate standard errors. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Dunnett's multiple test. C, basal diet group; 5S, basal diet containing 5% sea squirt group; 10S, basal diet containing 10% sea squirt group; 20S, basal diet containing 20% sea squirt group.

group were significantly lower than those of the basal diet group. The HDL-cholesterol concentration was not significantly different among the four groups.

Table 2 shows the liver lipid contents. The liver triglyceride content in the group fed 20% of the sea squirt was significantly lower than that in the group fed the basal diet. The liver cholesterol and phospholipid contents were not significantly different among the four groups.

The serum and liver TBARS values are also shown in Table 2. The serum TBARS value did not differ among the four groups. The liver TBARS value was gradually enhanced by the sea squirt feeding, and the value was significantly higher in the 20% sea squirt diet feeding group than in the basal diet group.

DISCUSSION

Feeding of the dietary sea squirt muscle decreased the serum total cholesterol, (VLDL+LDL)-cholesterol, triglyceride, phospholipid, and NEFA concentrations in a dose-dependent manner in rats, and these serum lipid concentrations in the group fed 20% of the sea squirt were significantly lower than those in the group fed the basal diet.

The mechanisms responsible for the observed decrease in these serum lipid concentrations that occurs as a result

Table 2. Cholesterol, triglyceride and phospholipid contents of liver, and thiobarbituric acid-reactive substance (TBARS) values of serum and liver in rats fed basal or sea squirt containing diet[1].

Measurement	Control[2]	5S	10S	20S
Liver lipid content (μ mol/g of liver)				
Cholesterol	5.15 ± 0.35	5.01 ± 0.19	4.93 ± 0.28	4.57 ± 0.18
Triglyceride	7.52 ± 0.46 [a]	5.89 ± 0.52 [ab]	5.99 ± 0.56 [ab]	5.00 ± 0.68 [b]
Phospholipid	32.3 ± 0.8	32.0 ± 0.5	32.7 ± 0.4	33.3 ± 1.0
TBARS				
Serum (μ mol/L)	3.69 ± 0.19	4.01 ± 0.22	4.17 ± 0.31	3.76 ± 0.24
Liver (nmol/g of liver)	107.4 ± 7.2 [a]	122.4 ± 8.2 [a]	193.3 ± 37.7 [a]	354.7 ± 62.5 [b]

[1] Each value represents the mean ± SEM for seven rats. Values not sharing a common letter are significantly different at $p < 0.05$ by one-way analysis of variance followed by Dunnett's multiple test.

[2] Definition of group names: Control, basal diet group; 5S, basal diet containing 5% sea squirt muscle group; 10S, basal diet containing 10% sea squirt muscle group; 20S, basal diet containing 20% sea squirt muscle group.

of dietary sea squirt remain to be determined in the present study. Sea squirt contains a high level of taurine (1.89g/100g) or a high ratio of n-3 polyunsaturated fatty acids as compared with other animal foods. It is reported that taurine has a serum cholesterol lowering effect.[15] The n-3 polyunsaturated fatty acids in seafoods, such as eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA), reduce a serum cholesterol concentration and suppress a cholesterol biosynthesis in the liver.[16] The serum triglyceride concentration also decreased by means of EPA and DHA. An enhancement of the activity of lipoprotein lipase, hydrolytic enzyme of triglyceride in serum, by dietary fish oil[17] and an inhibition of the synthesis and secretion of triglyceride by EPA[18] were observed. The EPA and DHA also decreased the serum NEFA concentration, and this serum lipid lowering effect is related, at least in part, to an elevation of fatty acid oxidation (β-oxidation).[19] The contents of EPA and DHA are high in the sea squirt oil as well as in other seafoods (the ratio of EPA and DHA to all fatty acids are 24.4% and 11.7%, respectively). Thus, these serum lipid lowering actions in the present study might have been related to taurine or EPA and DHA.

The liver TBARS value was elevated by 20% of the dietary sea squirt feeding. The TBARS value is an index of lipid peroxidation in serum or tissues. The polyunsaturated fatty acids are oxidized easily. The ratio of polyunsaturated fatty acids to all fatty acids in seafoods is higher than that in other animal foods. Liver lipid peroxidation might have been promoted by the sea squirt feeding, so a large increase in the liver TBARS value by feeding of 20% of the sea squirt might have been seen in the present study.

Sea squirt contains a high amount of minerals. In particular, the content of sodium in the sea squirt is higher than in other seafoods. Minerals are assumed to have little affect on lipid metabolism, so, these serum lipid lowering effects seen in the present study might have little or no relation to the amount of sodium in the dietary sea squirt.

In conclusion, the serum total cholesterol, (VLDL+LDL)-cholesterol, triglyceride, phospholipid, and NEFA concentrations in the group fed 20% of the sea squirt were significantly lower than those in the basal diet group. It is possible that the dietary sea squirt may in some way affect this lipid concentration lowering. It remains unknown what ingredients exert these serum lipid concentration lowering actions. Further studies are needed to clarify this point.

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和文要旨

ラットの血清および肝臓脂質レベルに対する食餌ホヤの作用

川　崎　雅　志

食餌ホヤに食品および栄養機能があるかどうかを検討するため，ホヤを摂取したラットの血清脂質濃度および肝臓脂質含量に対する作用について検討した。ラットには基本食あるいは5%，10%，20%のホヤ筋肉部分を含む実験食を与えた。血清総コレステロール，超低密度リポたんぱく質 (VLDL) + 低密度リポたんぱく質 (LDL) コレステロール，トリグリセリド，リン脂質および遊離脂肪酸濃度がホヤ摂取量の増加に従って徐々に低下し，20%摂取によって，基本食に比べて有意に低下した。血清高密度リポたんぱく質コレステロール濃度には4群間で差はみられなかった。肝臓トリグリセリド含量が20%ホヤの摂取により有意に低下した。肝臓コレステロールおよびリン脂質含量に4群間で有意な違いはみられなかった。血清チオバルビツール酸反応物質 (TBARS) 値に4群間で有意な差はなかった。肝臓TBARS値が20%ホヤの摂取により有意に上昇した。これらの結果より，ホヤ筋肉部分の摂取により血清総コレステロール，(VLDL+LDL)-コレステロール，トリグリセリド，リン脂質および遊離脂肪酸といった血清脂質の濃度を低下させる作用のあることが示唆された。

キーワード：食餌ホヤ，血清脂質濃度，ラット